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Research Article

Microbial Load of Some Medicinal Plants Sold in Some Local Markets in Abeokuta, Nigeria

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Abstract

***Purpose**: To evaluate the microbial load on 17 randomly selected plant samples from 60 ethnobotanically collected medicinal plants from five local markets in Abeokuta, Ogun State, Nigeria.*
***Method:** The pour plate method was used to cultivate serially diluted portions of the medicinal plant samples investigated. Enumeration of bacteria was carried out on nutrient agar (NA) while that of fungi was effected on Sabouraud agar (SA).*
***Results:** The identified microbial isolates include 12 bacterial and 6 fungal genera. The mean heterotrophic bacteria counts of the different herbal samples ranged from 1.3 × 10^5 cfu/g (Cnestis ferruginea) to 6.7 × 10^6 cfu/g (Daniellia oliveri), while total fungal propagule counts ranged from 0.0 × 10^1 cfu/g (Terminalia superba, Cola gigantea, Rauwolfia vomitoria, Zingiber officinale and Argemone mexicana) to 7.1 × 10^6 cfu/g (Nesogordonia papaverifera). The synopsis and frequency (prevalence rate) of microbial species isolation showed that Bacillus spp. (82.4 %) and Mucor spp. (47.1 %) had the highest prevalence rates among bacteria and fungi, respectively.*
***Conclusion:** The findings from this study emphasized the need for constant quality assessment of herbal drugs on sale in order to ensure the production of therapeutic products suitable for human consumption.*

***Keywords:** Microbial load; Medicinal plants; Local markets; Abeokuta; Nigeria*

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INTRODUCTION

In the past, medicinal plants was the first line of treatment known to man and traditional medicinal practice remain an important part of the primary healthcare delivery system in most of the developing world [1]. According to a World Health Organization (WHO) survey, about 70 – 80 % of the world's populations, particularly in the developing countries, rely on non-conventional medicines, mainly from herbal sources, for their primary healthcare [2].

Generally, plants constitute a major source of orthodox medicines and the presence of plant secondary metabolites has been attributed for most plants' therapeutic activities [3,4]. Phytomedicines have shown great promises in the treatment of intractable infectious diseases [5]. The local uses of plants and their products in healthcare are much higher in those areas with little or no access to modern healthcare services [6].

The global and national markets for medicinal herbs have been growing rapidly and significant economic gains are being realized with global sales of herbal products totalling an estimated US$60 million in 2000 [7]. However, the current global market for herbal medicines stands at over US$62 billion annually [8]. The sale of herbal medicine is expected to reach an annual average growth rate of 6.4% [9]. Several countries, despite their abundant tropical forests, earn nothing significant from the sector. For example, Nigeria is not on the list of countries that have a stake in the over $60 billion generated from herbs globally. It is at the moment generating 0.001 per cent of the revenue from herbs [10]. The local markets in Abeokuta form an integral part of the life of the people. Medicinal plants traders in these markets sell large amounts of plants to indigenes and visitors who seek their help. They mostly sell the plants (barks, roots, stems and leaves) in dried forms.

Again, market surveys are an efficient means of acquiring data on local values and conservation status of indigenous species [11]. An understanding of the market profile, social economic attributes influencing trade, species traded and impact of trade on plant pollution is critical for effective resources management [12]. The safety and quality of raw medicinal plant materials and finished products depend on factors that may be classified as intrinsic (genetic) or extrinsic (environment, collection methods, cultivation, harvest, post harvest processing, transport and storage practice [7].

It is against this backdrop that the microbial loads of some of the ethnobotanically collected plants in Abeokuta, Nigeria were carried out.

EXPERIMENTAL

Study Area

The study was carried out in the city of Abeokuta, Ogun State, Nigeria. Abeokuta is located within longitude $3^{0}2^{1}$ East and Latitude $7^{0}1^{1}$ North. The markets surveyed were Omida, Itoku, Adatan, Kuto and Lafenwa markets. Abeokuta lies within the tropical rainforest area of Nigeria with a population of about one million people and is surrounded by rocks [13].

Collection and identification of plant samples

The medicinal plant samples were bought from the five local markets surveyed in Abeokuta and aseptically collected with sterile cloves into sterile bags. The samples were immediately kept in clean, cool and dry baskets.

Most of the plant samples were identified by one of the authors (MI) while the others were identified on the basis of their local names and standard texts [14, 15]. Voucher specimens of the plants were kept in the Department of Plant Biology and

Biotechnology Herbarium, University of Benin, Nigeria.

Preparation and sterilization of media and samples

The media used were nutrient agar (NA) and Sabouraud agar (SA) for enumeration of bacteria and fungi, respectively. They were prepared according to the manufacturer's guide and sterilized in an autoclave at 121°C for 15 min. The dried plant samples were ground into fine particles under aseptic conditions in a surface sterilized laboratory bench. The grater was washed, dried and sterilized before use. After grinding, the samples were placed in different sterile universal containers and labelled accordingly.

Microbial analysis of plant samples

The pour plate method was used to cultivate serially diluted portions of the medicinal plant samples under investigation. Enumeration was carried out on nutrient agar (NA) for bacteria and on Sabouraud agar (SA) for fungi. Triplicate plates of appropriate dilutions were prepared. The NA plates were incubated at 37 ^{0}C for 24 - 48 h for bacterial growth while SA plates were incubated at room temperature (28 ± 2^{0}C) for 48 – 72 h for fungal growth. The developed microbial colonies were counted and computed as colony forming units per gram (cfu/g) of plant material. The colonies were purified, isolated and stored for morphological and biochemical characterization. These were further identified with to the aid of Bergey's Manual of Determinative Bacteriology [16] for bacteria and Illustrated Genera of Imperfect Fungi [17] for fungi.

Statistical analysis

The results were expressed as mean values ± SEM of three replicates of the total heterotrophic bacteria and fungi (cfu/g) contained in each plant sample screened. The data were analysed using Students t-test with the aid of SPSS 10 software package. The level of significance was set at 0.05. The prevalence rate of the microbial species was also computed.

RESULTS

The results of the microbial load of the different plant materials are presented in Tables 1 - 4. No significant difference was recorded in both the bacterial and fungal counts (Tables 1 and 2). The mean heterotrophic bacteria counts of the different herbal samples ranged from 1.3×10^5 cfu/g (*Cnestis ferruginea*) to 6.7×10^6 cfu/g (*Daniellia oliveri*) (Table 1), while Table 2 reveals that the total fungal propagule counts ranged from 0.0×10^1 cfu/g (*Terminalia superba, Cola gigantea, Rauwolfia vomitoria, Zingiber officinale* and *Argemone mexicana*) to 7.1×10^6 cfu/g (*Nesogordonia papaverifera*). The identified microbial isolates consist of 12 bacterial genera and 6 fungal genera as shown in Tables 3 and 4, respectively. The synopsis and frequency (prevalence rate) of the microbial species isolation showed that *Bacillus spp.* (82.6 %) and *Mucor spp.* (47.1 %) had the highest prevalence rates among bacteria and fungi, respectively. The least frequently isolated bacterial species were *Arizoma* spp., *Diphtheroids*, *Escherichia coli*, *Proteus* spp., *Streptococcus* spp. and *Pseudomonas aeruginosa* with a prevalence rate of 5.9 %. The figure was the same for the fungal species (*Absidia* spp., *Rhizopus nigrican* and *Saccharomyces cerevisiae*) with the least prevalence rate.

DISCUSSION

Samples cultured on nutrient agar (NA) were observed to have a large growth of bacterial species while samples cultured on Sabouraud agar (SA) were observed to give few results as fungal species did not grow on some of the cultured samples, namely, *Terminalia superba*, *Cola gigantea*, *Zingiber officinale*, *Argemone mexicana* and *Rauwolfia vomitoria* (Tables 1 and 2). Contamination by microorganisms is

Table 1: Heterotrophic bacterial counts (mean ± SD, n= 3) of medicinal plant samples obtained from some open markets in Abeokuta, Nigeria

Plant sample	Voucher no.	Heterotrophic bacterial count (cfu/g)	Predominant bacterial species isolated
Aristolochia repens	BDHS 160	$5.4x10^5$±0.01	*Citrobacter* spp., *Klebsiella aerogenes* and *Bacillus subtilis*
Terminalia superba	BDHS 103	$2.3x10^6$±0.01	*Bacillus subtilis, klebsiella aerogenes* and *Arizoma spp.*
Angylocalyx oligophyllus	BDHS 139	$3.5x10^6$±0.01	*Bacillus subtilis, Citrobacter* spp and *Staphylococcus epidermidis*
Theobroma cacao	BDHS 130	$9.3x10^5$±0.01	*Staphylococcus epidermidis, Citrobacter* spp, *Bacillus subtilis* and *Serratia marcescens*
Nesogordonia papaverifera	BDHS 108	$6.3x10^6$±0.03	*Pseudomonas aeruginosa, Citrobacter* spp, *Bacillus* spp. and *Staphylococcus epidermidis*
Daniellia oliveri	BDHS 132	$6.7x10^6$±0.01	*Klebsiella aerogenes* and *Staphylococcus aureus*
Ficus capensis	BDHS 146	$5.0x10^5$±0.02	*Serratia marcescens, Citrobacter* spp. and *Staphylococcus epidermidis*
Treculia Africana	BDHS 117	$1.0x10^6$±0.01	*Klebsiella aerogenes*
Mondia whitei	BDHS 105	$1.1x10^6$±0.02	*Staphylococcus epidermidis* and *Bacillus subtilis*
Securinega virosa	BDHS 112	$4.3x10^5$±0.02	*Bacillus subtilis* and *Escherichia coli*
Euphorbia lateriflora	BDHS 126	$6.0x10^5$±0.01	*Proteus* spp., *Staphylococcus epidermidis* and *Bacillus subtilis*
Cola gigantean	BDHS 151	$4.5x10^6$±0.02	*Acinetobacter* spp. and *Bacillus subtilis*
Rauwolfia vomitoria	BDHS 149	$3.8x10^6$±0.01	*Bacillus subtilis*, *Pseudomonas aeruginosa* and *Streptococcus* spp.
Cnestis ferruginea	BDHS 123	$1.3x10^5$±0.01	*Bacillus subtilis, Pseudomonas aeruginosa* and *Diphtheriods*
Zingiber officinale	BDHS 110	$2.0x10^6$±0.02	*Acinetobacter* spp., *Pseudomonas aeruginosa* and *Bacillus subtilis*
Argemone Mexicana	BDHS 143	$2.2x10^6$±0.02	*Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa*
Heliotropium indicum	BDHS 138	$3.0x10^5$±0.01	*Bacillus subtilis* and *Staphylococcus epidermidis.*

influenced by the environment, improper handling and storage of medicinal plants. One of the major shortcomings of herbal preparations in developing countries is the unhygienic condition under which they are produced [18]. In the present study, it was observed that herbal remedies were not sterile.

This may be due to lack of proper storage facilities and sales infrastructure within the markets visited, hence the plant materials failed to resist contamination [19]; where dispensing hygiene is not good, contamination may be even worse [20].

Inadvertent contamination by microbial or chemical agents during processing could also caused deterioration, thereby compromising safety and quality, and rendering the medicinal plant material less effective and possibly harmful to the consumer [21]. Spoilage of medicines involve basically, initial or early pioneer invaders of biodegrading microorganisms, which prepare the way for later invaders that biodegrade complex

nutrient, thus altering the surrounding pH and increasing moisture content [22]. The presence of lipolytic mould such as *Penicillium sp* and *Aspergillus niger* (Table 4) is of great concern as they have been implicated in food poisoning [23].

Table 2: Total fungal propagule counts (mean ± SD, n= 3) of medicinal plant materials obtained from some open markets in Abeokuta, Nigeria

Plant sample	Voucher no.	Fungal count (cfu/g)	Predominant fungal species isolated
Aristolochia repens	BDHS 160	$3.1 \times 10^6 \pm 0.04$	*Aspergillus fumigatus* and *Absidia* spp.
Terminalia superb	BDHS 103	NIL	No growth
Angylocalyx oligophyllus	BDHS 139	$7.5 \times 10^5 \pm 0.03$	*Mucor* spp.
Theobroma cacao	BDHS 130	$4.6 \times 10^6 \pm 0.04$	*Aspergillus fumigatus* and *Penicillium* spp.
Nesogordonia papaverifera	BDHS 108	$7.1 \times 10^6 \pm 0.01$	*Aspergillus niger* and *Mucor* spp.
Daniellia oliveri	BDHS 132	$4.7 \times 10^5 \pm 0.03$	*Aspergillus ochraeous*
Ficus capensis	BDHS 146	$5.7 \times 10^4 \pm 0.01$	*Mucor* spp.
Treculia Africana	BDHS 117	$1.7 \times 10^6 \pm 0.01$	*Saccharomyces cerevisiae*
Mondia whitei	BDHS 105	$8.5 \times 10^5 \pm 0.02$	*Mucor* spp.
Securinega virosa	BDHS 112	$7.1 \times 10^5 \pm 0.02$	*Mucor* spp. and *Penicillium* spp.
Euphorbia lateriflora	BDHS 126	$3.6 \times 10^6 \pm 0.02$	*Mucor* spp.
Cola gigantean	BDHS 151	NIL	No growth
Rauvolfia vomitoria	BDHS 149	NIL	No growth
Cnestis ferruginea	BDHS 123	$3.5 \times 10^6 \pm 0.01$	*Mucor* spp.
Zingiber officinale	BDHS 110	NIL	No growth
Argemone Mexicana	BDHS 143	NIL	No growth
Heliotropium indicum	BDHS 138	$6.0 \times 10^6 \pm 0.03$	*Rhizopus nigrican* and *Mucor* spp.

Table 3: Synopsis and frequency (prevalence rate) of isolation of bacterial isolates from herbal plant samples obtained from some open markets in Abeokuta, Nigeria

Bacterial isolate	Prevalence rate of isolation (%)
Acinetobacter spp.	11.77
Arizoma spp.	5.88
Bacillus subtilis	82.35
Citrobacter spp.	29.41
Diphtheriods	5.88
Escherichia coli	5.88
Klebsiella aerogenes	17.65
Proteus spp.	5.88
Pseudomonas aeruginosa	29.41
Serratia marcescens	11.77
Staphylococcus epidermidis	41.18
Staphylococcus aureus	11.77
Streptococcus spp.	5.88

Table 4: Synopsis and frequency (prevalence rate) of isolation of fungi from herbal plant samples obtained from some open markets in Abeokuta, Nigeria.

Fungal isolates	Prevalence rate of isolation (%)
Absidia spp	5.88
Aspergillus fumigatus	11.77
Aspergillus niger	11.77
Aspergillus ochraeous	5.88
Mucor spp.	47.06
Penicillium spp.	11.77
Rhizopus nigrican	5.88
Saccharomyces cerevisiae	588

CONCLUSION

The findings from this study reiterate the need for constant quality assessment of herbal materials in the market in order to ensure that medicinal plant materials and products are suitable for human

consumption. Medicinal plants sold in markets should be placed in clean sterile baskets or suitable hygienic packs. The moisture content of the plant materials should always be maintained at minimal levels to reduce the rate of microbial proliferation.

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